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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/430,175	10/29/1999	STEPHEN A. LESKO	CW-304	6875

7590 07/21/2004

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EXAMINER	
CANELLA, KAREN A	

ART UNIT	PAPER NUMBER
1642	

DATE MAILED: 07/21/2004

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	09/430,175	LESKO ET AL.	
	Examiner	Art Unit	
	Karen A Canella	1642	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☐ Responsive to communication(s) filed on *Mar 22, 2004*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☐ Claim(s) *1,2,9-25,28,31-41,44-56,58-61,64-66 and 68-70* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☐ Claim(s) *1,2,9-25,28,31-41,44-48,51-56,58,59,64-66 and 68-70* is/are rejected.

7)☐ Claim(s) *49,50,60 and 61* is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

Claims 1, 2, 11, 12, 15-25, 28, 34-39, 43, 45, 48-56, 59-61, 64-66 and 68-70 have been amended. Claims 29 and 30 have been canceled. Claims 1, 2, 9-25, 28, 31-41, 44-56, 58-61, 64-66 and 68-70 are pending and under consideration.

After review and reconsideration, the finality of the Office action of Dec 22, 2003 is withdrawn.

The text of those sections of Title 35, U.S. Code not included in this action can be found in a prior Office Action.

Claims 1, 2, 9, 10, 11, 12, 13-25, 28, 33-41, 43-46, 51, 53-56, 59, 64, 65, 66, 68, 69, 70 are rejected under 35 U.S.C. 103(a) as being unpatentable over Ts'o (Urology, 1997, Vol. 49, pp. 881-885) in view of Galbraith et al (reference AS15 of the IDS filed January 28, 2001) and Waggoner et al (reference AT15 of the IDS filed January 28, 2001).

Claim 1 is drawn to a method of characterizing single circulating epithelial cancer cells obtained from about 5ml to 75ml blood comprising concurrently measuring multiple cellular markers expressed in said cells using fluorescence microscopy, wherein said fluorescent probes comprise five fluorescent probes. Claim 2 embodied the method of claim 1 wherein said cells are isolated by density gradient centrifugation from a sample containing said cells, said isolated cells are adhere onto a surface and fixed with a fixative solution, and said subsurface containing cells for characterization is incubated with said probes, wherein each probe reacts with a marker of said cells and any probe binding with a marker is examined by a microscope equipped with an optical filter set for identification of each specific marker. Claim 9 embodies the method of claim 2 wherein the surface for cell adherence is a microscope slide. Claim 10 embodies the method of claim 2 wherein the fixative is selected from the group consisting of paraformaldehyde, formaldehyde, alcohol or acetone. Claim 56 embodies the method of claim 2 wherein cells are further isolated by a negative selection process. Claim 11 embodies the method of claim 1 wherein one or more of said probes is covalently linked to a fluorescent compound that emits a wavelength of light to create a fluorescent probe that binds to a cellular marker.

Claim 12 embodies the method of claim 11 wherein one or more of said fluorescent probe is selected from other probes with minimal overlapping emission spectra for concurrent use in characterizing said cells. Claims 13-25 are drawn to the method of claim 12 wherein the fluorescent compounds have various emission spectra that can be distinguished from each other. Claim 28 is drawn to known fluorescent labels. Claim 33 embodies the method of claim 1 wherein the probes comprise multiple fluorescence probes that emit light of different wavelengths with minimal interference between the wavelengths. Claim 34 embodies the method of claim 1 wherein one or more of said probes is directed to a cellular target and is not a nucleic acid. Claim 35 embodies the method of claim 34 wherein one or more of said probes comprises a protein or peptide. Claim 36 embodies the method of claim 35 wherein one or more of said probes in an antibody. Claim 37 embodies the method of claim 1, wherein one or more of said probes is a nucleic acid directed to a cellular target. Claims 38 and 38 embody the method of claim 37 wherein one or more of said probes comprises DNA and RNA, respectively. Claim 40 embodies the method of claim 1 wherein said probes comprises (I) probes which are directed to a cellular target and are not a nucleic acid, (II) probes which are a nucleic acid directed to a cellular target or (III) a combination of (I) ad (II). Claim 41 embodies the method of claim 40 wherein said probes are selected from the group consisting of identification probes, proliferation probes, cell cycle arrest probes, oncogenes and hormonal probes. Claims 43 and 44 embody the method of claim 40 wherein the probes comprise an epithelial cell-specific probe and a tissue specific probe, respectively. Claim 45 embodies the method of claim 1 wherein said cells are obtained form a mammal. Claim 46 specifies that the mammal of claim 45 is a human. Claim 51 embodies the method of claim 1 wherein one or more of said cellular markers is an antigen.

Claim 53 is drawn to a method of characterizing a single epithelial cancer cell preparation obtained from about 5 to 75 ml blood, said method comprising adhering a circulating epithelial cancer cells to be characterized onto a surface, fixing said cell preparation with a fixative solution, incubating said cell surface containing fixed cells with multiple probes directed to desired cellular markers, wherein said multiple probes have the ability to fluoresce when excited at different wavelengths, and examining the cells by fluorescence microscopy for identification of positive cells for each selected marker by concurrent measurement of multiple cellular

markers, wherein said cancer cells are isolated from a body fluid using a negative selection process, wherein said circulating epithelial cancer cells are obtained and wherein said fluorescent probes comprise five fluorescent probes. Claim 55 embodies the method of claim 53 wherein said epithelial cancer cells are isolated by density gradient centrifugation from a sample containing cells, said isolated cells are adhered onto a surface and fixed with a fixative solution, and said surface containing cells for characterization is incubated with said probes, wherein each probe reacts with a marker of the single cell, and any probe binding with a marker is examined by a microscope equipped with an optical filter set of identification of each individual marker

Claim 54 is drawn to a method of establishing a characterization profile of a circulating epithelial cancer cell obtained from about 5 ml to 75 ml blood comprising characterizing a single cell environment by concurrent measurement of multiple cellular markers using fluorescent probes, wherein said probes emit different wavelengths of light to distinguish multiple cellular markers expressed in the single cell using fluorescence microscopy and wherein said fluorescent probes comprise five fluorescent probes. Claim 59 embodies any one of claims 1, 53 and 54, wherein one or more of said circulating epithelial cancer cells is a prostatic cancer cell. Claims 64, 65 and 66 embody the method of any one of claims 1, 53 and 54 wherein one or more of said circulating epithelial cancer cell is obtained from about 5 to 25 ml of blood, 15-25 ml of venous blood and about 20 ml blood, respectively. Claims 68 and 69 embody the methods of claims 64 and 65, respectively, wherein one or more of said epithelial cancer cell is a prostatic cancer cell. Claim 70 embodies the method of any one of claims 1, 53 and 54, wherein said probe are selected from the group consisting of (a)tissue specific probes for determining the cellular origin of the cell, (b) probes specific for tumor cell markers, (c)probes specific for aneuploidy, (d) probes specific for cellular markers of proliferation, (e) probes specific for cellular markers of growth inhibition, (f) probes specific for cell-cycle arrest, (g) probes specific for cellular markers of apoptosis and (h) probes specific for hormonal receptors.

Ts'o teaches a method of characterizing single circulating prostate epithelial cancer cells obtained from about 20 ml blood (page 881, second column, lines 1-2 under the heading "Blood collection") comprising isolating a cellular fraction of the blood (page 883, first column, lines 5-7 under the heading "Prostate Cell detection From Patients with Advanced Prostate Cancer"),

and by means comprising isolating by density gradient centrifugation from blood page 882, first column, lines 1-11 under the heading "single Density Gradient Centrifugation"), a sample comprising prostate cancer cells, adhering the sample by contact with an alcohol fixative to a microscope slide page 882, first column, lines 1-4 under the heading "Identification of Isolated Cells"), and incubating the adhered, fixed sample with a multiplicity of probes consisting of anti-PSA monoclonal antibody, polyclonal anti-prostatic acid phosphatase, diaminophenylindole (page 882, second column, lines 1-4) and DNA probes specific for the centromeric regions of chromosomes 7 and 8 (page 882, second column, lines 15-17) wherein each probe reacts with a marker of the prostate cell, and the adhered, fixed sample after contact with the probes is examined by a fluorescent microscope equipped with optical filters for the identification of each specific probe (page 882, second column, lines 4-8). Ts'o et al disclose that the microscope was equipped with filters to allow for the differential detection of various fluorescent dyes (page 882, second column, lines 4-8) as evidenced by Figure 1 which illustrates that green represents immunoreaction with the anti-PS antibody; yellow represents immunoreaction with the anti-PsAP antibody and blue indicates diamidinophenylindole, as well as blue representing chromosome 7 centromere and red representing chromosome 8 centromere. It is apparent from figure 1, legend (E) that human prostate cancer cells were stained green by monoclonal antibody against PSA, blue for chromosome 7 centromere and red for chromosome 8 centromere, thus fulfilling the specific embodiments of multiple probes, concurrent measurement and minimal overlapping emission spectra, thus fulfilling the specific embodiments of claims 1, 2, 9-12, 33, 53-55. Ts'o further teaches the specific embodiments of claims drawn to a probe directed to a cellular target that is not a nucleic acid, a probe that is epithelial cell specific and tissue specific (PSA and PsAP), a probe that is an antibody (anti-PSA, anti PsAP), a probe comprising DNA and RNA (probe for centromeres on chromosomes 7 and 8) which contains RNA (tuna as part of the in situ hybridization buffer), probes specific for tumor cell markers, probes for determining the cellular origin of the cells (prostate), probes specific for aneuploidy (Centromere chromosomes 7 and 8, page 883, first column, last sentence). Ts'O teaches the concurrent use of fluorescent labeled antibody probes for PSA and PsAP along with diaminophenylindole which stains nuclear DNA. Ts'O teaches the concurrent use of probes for chromosome 7, chromosome 8 and diaminoophenylindole for the staining of nuclear DNA. Thus Ts'o et al teach the

concurrent measurement of three probes including probes to surface antigens, and the separate concurrent measurement of the concurrent measurement of three probes including probes for specific chromosomes. Ts'o does not teach the concurrent measurement of five fluorescent probes, nor does Ts'o et al specifically teach that the fluorescent probes are chosen to have minimal overlapping emission spectra for concurrent use in characterizing cells.
Galbraith et al (reference AS15 of the IDS filed January 28, 2001) teach that fluorescent imaging cytometry is superior to flow cytometry because the method is not limited by the number of fluorophores which may be distinguished (page 592, first column, second full paragraph). Galbraith et al teach that the selection of fluorophores which have spectral properties which are sufficiently separated as to be measured independently, and optimization of the excitation and emission filters in the fluorescent microscope (page 562, first column, third full paragraph). Waggoner et al (reference AT15 of the IDS filed January 28, 2001) teach multicolor analysis of populations of single cells and a microscope slide surface containing cells (page 499, first column, "Multicolor Immunophenotyping") by quantitative fluorescent imaging (page 499, second column, last paragraph). Waggoner et al teach spectroscopic properties of selected probes (Table 1) which include fluorescent haptens, reagents for determining DNA/RNA content, and probes for membrane location and fluidity, and physiologic probes sensitive to intracellular calcium and pH.

It would have been *prima facie* obvious at the time the claimed invention was made to label the probes used for the immunofluoresce detection of PSA and PsAP along with the probes used for the in situ hybridization and the probe for the nuclear DNA with fluorescent markers having spectral properties which can be measured independently and use said labeled probes for the concurrent measurement of PSA, PsAP, chromosome 7, chromosome 8 and nuclear DNA. One of skill in the art would have been motivated to do so by the teachings of Galbraith et al on the technology for distinguishing fluorophores having different spectral properties and the teachings of Waggoner et al on the known spectroscopic properties of selected probes, One of skill in the art would have been motivated to measure the five markers concurrently rather than in two steps in order to reduce the processing time for each sample.

The rejection of claims 1, 2, 9, 10, 11, 12, 13-25, 28, 33-41, 43-46, 51, 53-56, 58, 59, 64, 65, 66, 68, 69, 70 under 35 U.S.C. 103(a) as being unpatentable over Ts'o (Urology, 1997, Vol. 49, pp. 881-885) and Galbraith et al (reference AS15 of the IDS filed January 28, 2001) and Waggoner et al (reference AT15 of the IDS filed January 28, 2001) as applied to claims 1, 2, 9, 10, 11, 12, 13-25, 28, 33-41, 43-46, 51, 53-56, 59, 64, 65, 66, 68, 69, 70, above and in further view of Ross (US 5,674,694) is maintained for reasons of record.

Claim 58 embodies the method of claim 2 wherein cells are further isolated by a positive selection process wherein the specific cell type is selected from a heterogeneous mixture of cells by an antibody that specifically binds to a specific cell type. The combination of Ts'o et al and Galbraith et al and Waggoner et al render obvious a negative selection method. Non of the aforesaid references teach a negative selection method followed by a positive selection method. Ross teach that tumor cells may be isolated from hematopoietic cells by either positive selection or negative selection, and if desired, the process used to enrich the tumor cell population may be preformed more than once or in any combination where appropriate (column 8, line 66 to column 9, line 13)..

It would have been be *prima facie* obvious o one of ordinary skill in the art at the time the invention was made, to further isolate the cancer cells by a positive selection method. One of ordinary skill in the art would have been motivated to do so with a reasonable expectation of success by the teachings of Ross on the usefulness of both positive and negative selection methods in combination for the isolation of tumor cells.

The rejection of claims 1, 2, 9, 10, 11, 12-25, 33-41, 43-47, 51, 53-56, 59, 64, 65, 66, 68, 69, 70 under 35 U.S.C. 103(a) as being unpatentable over Ts'o (Urology, 1997, Vol. 49, pp. 881-885) and Galbraith et al (reference AS15 of the IDS filed January 28, 2001) and Waggoner et al (reference AT15 of the IDS filed January 28, 2001) as applied to claims 1, 2, 9, 10, 11, 12, 13-25, 28, 33-41, 43-46, 51, 53-56, 59, 64, 65, 66, 68, 69, 70, above in further view of Takeda et al (Cancer, 1996, Vol. 77, pp. 934-940, cited in a prior Office action) is maintained for reasons of record.

Claim 47 embodies the method of claim 40 wherein said probes are used to detect a hormone receptor. Claim 48 embodies the method of claim 37 wherein the hormone is an androgen. Claim 52 embodies the method of claim 51 wherein said cellular marker is a receptor.

The combination of Ts'o and Galbraith et al and Waggoner et al render obvious the specific embodiments of claims 1, 2, 9, 10, 11, 12, 13-25, 28, 33-41, 43-46, 51, 53-56, 59, 64, 65, 66, 68, 69, 70 for the reasons set forth above. Ts'o et al do not teach the detection of a hormone receptor or an androgen receptor.

Takeda et al teach the immunohistochemical detection of the androgen receptor in patients with prostate carcinoma.

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to include a fluorescent probe for the androgen receptor in the imaging cytometry of circulating prostatic epithelial cells as taught by Ts'o et al. One of skill in the art would have been motivated to do so with a reasonable expectation of success by the teachings of Takeda et al on the prognostic importance of androgen receptor status in prostate cancer patients.

The objection to claims 49, 50, 60 and 61 for being dependent on a rejected base claim is maintained for reasons of record.

Applicant argues that the prior rejections were fault as they combination of references did not render obvious the concurrent measurement of five fluorescent probes. It is noted that this limitation has been introduced into independent claims 1, 53 and 54. The examiner contends that it would have been obvious to concurrently measure fluorescent markers for each of PSA, PsAP, nuclear DNA, chromosome 7 and chromosome 8, wherein each of the markers have spectral properties which are sufficiently separated as to be measured independently because this would result in a faster acquisition of data and allow for the processing of more samples in a given time. It is obvious that the technology for the concurrent measurement of specific fluorescent markers was known in the art by the teachings of Galbraith et al and Waggoner et al teach numerous fluorescent probes which could be used in said method.

It is noted that method claims drawn to the detection of circulating epithelial cells other than prostate cells would be allowable because of the blood volume limitation.

All other rejections and objections not specifically set forth in this Office action are withdrawn.

THIS ACTION IS MADE FINAL. Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Karen A Canella whose telephone number is (571)272-0828. The examiner can normally be reached on 10 a.m. to 9 p.m. M-F.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Christina Chan can be reached on (571)272-0841. The fax phone number for the organization where this application or proceeding is assigned is 703-872-9306.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

Karen A. Canella, Ph.D.
7/19/2004